corresponding to the cell membrane. Within this cell membrane region, SPR images show punctate regions of high reflectivity that are putatively the cellular focal adhesions. Focal adhesions are known to be regions of high protein density that reside at the cell-substratum interface. Comparing SPR analyzed images with fluorescent antibody stain for Vinculin in rat aortic smooth muscle cells reveals a similar distribution of focal adhesion size and normalized intensity. Subsequently, comparing SPR images for several cell types reveals a distinct difference in focal adhesion intensity levels corresponding to a difference in protein density. In general, a positive correlation between focal adhesion size and intensity level is revealed by SPR imaging. Also, visualized around the cell edge is extracellular deposited material that corresponds to an approximate monolayer coating that extends beyond the cell, up to 40 μm around the cell periphery, indicating the ability to visualize cell secreted modification of the substrate for specific cell types.

142-Plat
Correlation Functions Provide a Universal Framework for Quantitative Analysis of Localization-Based Super-Resolution Microscopy Images
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Super-resolution images reconstructed from single molecule coordinates reveal cellular structures close to the macromolecular scale. However, a widely applicable analysis tool that can extract a quantitative description and biophysical parameters from these images is still unavailable. Here, we propose a universal analysis concept for coordinate-based super-resolution images using correlation functions. We demonstrate how this analysis can quantify diffusion, localization precision, distance and co-localization. Moreover, we developed a framework for averaging multiple images by non-linear transformations to reveal structures with much higher resolution. First, we show that the commonly used pair-distance histogram is mathematically equivalent to pixel-based correlation analysis. Therefore, many strategies of pixel-based image correlation spectroscopy can also be applied to coordinate-based super-resolution images. In particular, the computational complexity of pair-distance calculations can be decreased by pixel-binning the coordinate image and employing the Fast Fourier Transform algorithm.
Second, we demonstrate that the pair-distance histogram of molecule coordinates from consecutive frames allows measuring the diffusion coefficient of membrane lipids in live cells with high spatial resolution. Our analysis can handle extremely high fluorophore densities, in contrast to conventional single-particle tracking methods. The same frame-pair histogram can analogously be used to generate a map of molecule localization precision for super-resolution images, which is still elusive information.
Third, we show how coordinate-based correlation functions can be used for image alignment involving transformations beyond translation. For example, we rotationally aligned multiple images of the nuclear pore complex from yeast, which exposes the octagonal complex with high precision.
Finally, we propose a new correlation function, namely the point-set distance histogram, which allowed us to quantify both co-localization and distance of Clathrin to proteins of the Golgi apparatus, even though the super-resolution images were highly corrupted by background and two-color crosstalk.

143-Plat
Super-Resolution Imaging of Protein-Protein Interactions by Bimolecular Complementation of Photoactivatable Fluorescent Proteins
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Super-resolution microscopy, a powerful technique for determining protein localization, cannot discriminate between random colocalization of proteins and actual protein-protein interactions. Bimolecular fluorescence complementation, on the other hand, directly probes inter-molecular interactions, but has been classically limited by the diffraction limit. Here, we demonstrate that three photoconvertible fluorescent proteins (PA-FP) – PAmCherry1, Dendra2 and a Dronpa variant – support fluorescence complementation, and that split-PA-FPs can be used after complementation for photoactivated localization microscopy (PALM). We recovered photoconvertible fluorescent complexes by fusing split-PAmCherry1 to two interacting subunits of mammalian ATP-synthase. PALM images obtained in situ from such constructs were of quality similar to those obtained by labeling and imaging a single ATP-synthase subunit; however, in this scheme, each PALM event corresponded not only to a single ATP-synthase complex localized with ~20nm accuracy, but also, more precisely, to two subunits of that complex interacting within <10nm of each other.

Platform: Protein Gymnastics of Large-Scale Structural Rearrangements

144-Plat
Robustness of Rotary Catalysis Mechanism of F_1-ATPase
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Motor proteins convert chemical energy of ATP hydrolysis into mechanical force and movement, which play various physiological roles, such as organelle transport, muscular contraction, and energy synthesis. Among them, F_1-ATPase (F_1), rotary motor protein, has a unique feature to achieve the extremely high chemo-mechanical coupling efficiency and reversibility. To understand the energy coupling mechanism of F_1, extensive studies have been done for identifying the important interactions with ATP in the catalytic site. The interaction with phosphate moiety of ATP via three charged amino-acid residues (p-loop lysine, general base, and arginine-finger), which are well conserved in p-loop NTPases including motor proteins, is the most crucial for catalysis; e.g., upon the genetic depletion of these charged residues, the mutant F_1s do not show detectable catalytic activity. On the other hand, the interaction with base moiety of ATP via phenylalanine residues is crucial for binding of ATP to the catalytic site; e.g., upon the substitution of base group to uracil, the binding affinity to F_1 is extremely weakened. In the present study, we retested the competency of catalysis and force generation by using the charge-depleted mutants or uracil-substituted substrate (UTP) in the single-molecule rotation assay, which offers us to assess them with great sensitivity and preciseness. Surprisingly, all mutants showed the processive rotation with a constant rotary torque, even though the binding or hydrolysis rate of ATP was extremely slowed down by a factor of 10,000. Thus, the chemo-mechanical coupling mechanism of F_1 is found to be prominently robust; the catalysis is extremely tightly coupled to the torque generation, which probably contributes to its high efficiency and reversibility.

145-Plat
Electrostatic Basis of the Unidirectional Walking Motion in Myosin Molecular Motors
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Understanding the basis for the unidirectional motion of myosin motors on actin filaments require a quantitative energy based description of the overall functional cycle. Several experimental and theoretical studies have provided interesting insights on the functional cycle, but an understanding of the motor's unidirectionality from a non-phenomenological structure based free energy landscape is still missing. Here we use a coarse grained model of myosin V and generate a structure-based free energy surface of the largest conformational change, namely the transition from the post- to pre-power stroke movement. We also couple the observed energetics of ligand binding/hydrolysis and product release to that of the conformational surface to reproduce the energetics of the complete mechano-chemical cycle. It is found that the release in electrostatic free energy upon changing the conformation of the lever arm and the convertor domain from its post- to pre-power stroke states provides the necessary energy to bias the system towards the unidirectional movement of myosin V on actin filament. The free energy change of 11 kcal is also in the range of approximately 2-3 pN, which is consistent with the experimentally observed stalling force required to stop the motor completely on its track. The conformational-chemical coupling generating a successful power stroke cycle is believed to be conserved among most members of the myosin family, thus highlighting the importance of the previously unknown role of